A MODIFIED SYNTHESIS OF THIAMIN PYROPHOSPHATE AND DIHYDROXYACETONE MONOPHOSPHATE

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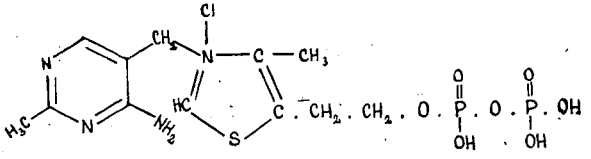
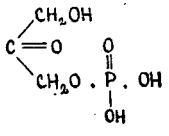
Improved procedures for synthesising the biochemically important materials, thiamin pyrophosphate and dihydroxyacetone monophosphate, are described. The former compound was successfully purified by precipitation with styphnic and phosphotungstic acids.

Organic phosphorus compounds, containing the O—P bond, may be divided, according to their biochemical behaviour, into two general categories: 1. Compounds containing so-called high-energy phosphate bonds and 2. those having low-energy phosphate bonds. A most typical representative of the first group is adenosine triphosphoric acid (ATP); the second group includes such compounds as, *e. g.*, the phosphates of glucose and fructose.

The objects of the syntheses described in this paper were thiamin pyrophosphate (TPP), which belongs to the group of esters containing high-energy bonds, and dihydroxyacetone monophosphate (DAP), a compound having low-energy phosphate bond.



TTF = TPF = thiamin trifoszfát



DOAF = dioxiacetonfoszfát

According to present knowledge, the pyrophosphoric acid ester of thiamin has an important role in biological decarboxylation processes; the compound is regarded

as the prosthetic group of carboxylase systems, therefore it is also named cocarboxylase [1]–[6]. On the other hand, in the opinion of other authors [7], this compound is important as a phosphate donor. Later it was found that TPP occured also in various animal tissues [8]. The role of TPP in decarboxylation and transketolation processes is dealt with in detail by WIESNER and VALENTS [9].

The biosynthesis of TPP may be readily effected from thiamin in the presence of yeast cells [10].

The chemical synthesis has the serious drawback that besides the required thiamin pyrophosphate or thiamin triphosphate (TTP) it gives various by-products, such as thiamin diphosphate (TDP), monophosphate (TMP), and possibly even thiamin tetraphosphate [11]. Several methods have been developed for eliminating by-product formation during the preparation of TPP and for the purification of the product [12]–[15].

We have applied a combination of WEIJLARD's purification procedure [13] with the method of preparation of BIGLINO and SEGRE [14]. The principle of our synthesis was heating of thiamin hydrochloride and phosphorus pentoxide in a medium of ortophosphoric acid and final purification of the product by precipitating with styphnic and phosphotungstic acids. Details of the procedure are given in the experimental part.

Dihydroxyacetone monophosphate (DAP), a component of the enzyme aldolase, may be synthesised in two ways.

1. Dihydroxyacetone is treated with phosphorus oxychloride in anhydrous quinoline at low temperatures. The product is finally isolated in the form of its calcium salt [16].

2. In the other synthesis, ethyl metaphosphate is used for introducing the phosphate group. The product is isolated in the form of its barium salt [17].

In the course of the present investigation both methods of preparation have been used and the details of the procedures established, as shown in the experimental part.

Experimental

Dihydroxyacetone: The product of the I. G. Farbenindustrie Aktiengesellschaft, Frankfurt a. Main, Höchst, was used.

Thiamin pyrophosphate

Phosphorus pentoxide (50 g) was dissolved at 110—120° C in 50 ml of 85% orthophosphoric acid and 13,0 g of thiamin hydrochloride was added in small portions. The addition was completed in about 30 minutes. The solution was allowed to stand 24 hours, then 60 ml of 2*N* phosphoric acid and 200 ml of water were added. After treating with decolourising carbon and filtration, anhydrous acetone was added until the solution became slightly turbid. The mixture was allowed to stand overnight in a refrigerator. A white crystalline precipitate was obtained which was filtered, and the mother liquor was again treated with acetone as described.

The first crop of crystals was dissolved in 2*N* hydrochloric acid, and the TPP precipitated again by treatment with acetone. A repetition of this procedure afforded a pure product with correct values of combustion analysis.

Final purification of the material could, however, be effected only by precipitation with styphnic acid (1,3-dihydroxy-2,4,6-trinitrobenzene) and with phosphotungstic acid. For this purpose, 1 g of the material was dissolved in 5 ml of water, 5 ml of 8% styphnic acid solution was added, then the mixture was treated with anhydrous acetone until it became turbid. A powdery precipitate separated on standing in a refrigerator, which was filtered and dried under infrared light.

1 g of the product obtained as described, was dissolved then in 50 ml of 2*N* hydrochloric acid, and precipitated with 20 ml of 25% phosphotungstic acid. The formed precipitate could be filtered off and analysis showed it to be identical with TPP.

Analysis: $C_{12}H_{19}O_7N_4SP.3\ H_2O$ (479,37) requires: C 33,85; H 4,46; N 13,17; P 14,58%. Found: C 33,97; H 5,02; N 13,34; P 14,50%.

Calcium dihydroxyacetone monophosphate

A solution of 1 g dihydroxyacetone in 30 ml anhydrous quinoline was cooled to -15 to $-20°$ C, and it was dropwise added to a cooled solution of 2 g $POCl_3$ in 10 ml quinoline. During the whole addition the mixture was kept at the temperature specified above. In about half an hour the material solidified to a thick crystalline mass. It was diluted with an equal volume of ice-water and 5 ml of 25% calcium acetate solution was added. Then the solution was neutralized by means of sodium hydroxide until it was only slightly acidic to litmus, centrifuged to separate the quinoline and calcium phosphate, and finally the calcium salt of dihydroxyacetone phosphate was precipitated by admixing three volumes of alcohol.

The purity of the product may be checked by analysing a sample for inorganic, hydrolysable and total phosphate contents. If the presence of significant amounts of dihydroxyacetone diphosphate is detected, the product may be purified by hydrolysis in 2 *N* HCl, and subsequent precipitation with alcohol.

Ethyl metaphosphate

Phosphorus pentoxide (200 g) was refluxed in excess diethylether, which had been previously dried over sodium, until the P_2O_5 was converted to a viscous syrup. This process required about 60 hours. The supernatant ether was separated, the material was dissolved in 400 ml of chloroform and filtered. The solution was refluxed for 5 hours in the steam bath, when the difficultly soluble components separated. After filtering from the solids, the ethyl metaphosphate was precipitated by the addition of two volumes of anhydrous ether.

A thick white syrup was obtained whicht tends to turn brown on standing. The material is not stable during storage. It can be stored for a while in chloroform, however on contact with air it turns rapidly brown in this case, too. If the solvent is separated from the freshly prepared syrup, the material may be used directly for the preparation of dihydroxyacetone monophosphate.

Barium dihydroxyacetone monophosphate

Crystalline dihydroxyacetone (0,945 g) was dissolved in a small amount of water and the solvent was repeatedly evaporated in an exsiccator over concentrated sulphuric acid, until the original weight was obtained. The procedure was repeated several times. The material was then thoroughly mixed with 0,9 g of ethyl metaphosphate syrup; this process resulted in the formation of a pure white material.

Unreacted ethyl metaphosphate was removed from the product by repeated extractions with ether.

After having evaporated the chloroform, the residue was dissolved in water and filtered. The filtrate was carefully neutralized with 15 ml of saturated $Ba(OH)_2$ solution. The mixture was allowed to stand overnight, and the small amount of precipitated barium phosphate was filtered.

The barium salt of dihydroxyacetone monophosphate was precipitated from the filtrate by the addition of anhydrous ethanol. Sometimes an amorphous precipitate was obtained; it could be transformed into crystalline material by dissolving in water and reprecipitating with ethanol.

Uniformity of the product was checked by paper chromatography, in butanol-acetic acid-water solvent with detection by ammonium molybdate and $SnCl_2$ [18].

When excess ethyl metaphosphate was employed, the principal product was dihydroxyacetone diphosphate.

When in the above procedure, neutralization was carried out by means of $Ca(OH)_2$ instead of $Ba(OH)_2$, calcium dihydroxyacetone phosphate was obtained. It has the advantage over the barium salt that it can be decomposed by as mild an agent as $1N$ oxalic acid; this behaviour is common with that of calcium salts of phosphate esters in general, e. g., calcium 1,6-fructose diphosphate. Centrifuging of the insoluble calcium oxalate and repetition of the procedure several times, gave the pure ester. On the other hand, decomposition of the barium salt requires the use of sulphuric acid, consequently in this case a certain degree of decomposition of the product may be involved, too.

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МОДИФИЦИРОВАННЫЙ СИНТЕЗ ТИАМИН ПИРОФОСФАТА И ДИОКСИ-АЦЕТОНФОСФАТА

Б. Маткович, П. Пэнзеш и Ш. Фельдеак

Известны различные методы для введения фосфатной группы или фосфатных групп в органические вещества. Здесь должны быть выдвинуты лишь два из них, служащие для фосфорилирования гидроксильных групп первичных спиртов. Различие между двумя методами состоит в том, что в то время как в случае диокси-ацетонфосфата образуется эфирная связь в случае тиамина создается пирофосфатная связь.